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# Non-stereospecific Transamination Catalyzed by Pyridoxal Phosphate-dependent Amino Acid Racemases of Broad Substrate Specificity

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Pyridoxal 5'-phosphate-dependent amino acid racemases of broad substrate specificity catalyze transamination as a side-reaction. We studied the stereospecificities for hydrogen abstraction from C-4' of the bound pyridoxamine 5'-phosphate during transamination from pyridoxamine 5'-phosphate to pyruvate catalyzed by three amino acid racemases of broad substrate specificity. When the enzymes were incubated with (4'S)- or (4'R)-[4'-<sup>3</sup>H]-pyridoxamine 5'-phosphate in the presence of pyruvate, tritium was released into the solvent from both pyridoxamine 5'-phosphates. Thus, these enzymes abstract a hydrogen non-stereospecifically from C-4' of the coenzyme in contrast to the other pyridoxal 5'-phosphate-dependent enzymes so far studied which catalyze the stereospecific hydrogen removal. Amino acid racemase of broad substrate specificity from *Pseudomonas putida* produced D- and L-glutamate from  $\alpha$ -ketoglutarate through the transamination with L-ornithine. Because glutamate does not serve as a substrate for racemization, the enzyme catalyzed the non-stereospecific overall transamination between L-ornithine and  $\alpha$ -ketoglutarate. The cleavage and formation of the C-H bond at C-4' of the coenzyme and C-2 of the substrate thus occurs non-stereospecifically on both sides of the plane of the coenzyme-substrate complex intermediate. Amino acid racemase of broad substrate specificity is the first example of a pyridoxal enzyme catalyzing non-stereospecific transamination.

**Keywords :** Amino acid racemase/ Stereochemistry/ Pyridoxal phosphate

Although enzymatic racemization of amino acid is apparently simple, consisting of a non-stereospecific rearrangement of the substrate  $\alpha$ -hydrogen, several different types of amino acid racemases are found in microorganisms. Aspartate racemase and glutamate racemase are independent of cofactors. Alanine racemase in several microorganisms, and amino acid racemases of broad substrate specificity of *Pseudomonas putida* depend

on pyridoxal 5'-phosphate (PLP). The reaction of amino acid racemase is initiated by transaldimination. In this step, PLP bound with the active-site lysyl residue through an internal Schiff base (Scheme I, A) reacts with a substrate to form an external Schiff base (B). The subsequent  $\alpha$ -hydrogen abstraction results in the formation of a resonance-stable anionic intermediate (C). If the reprotonation occurs at C-2 of the substrate moiety on the

## MOLECULAR BIOFUNCTION - Molecular Microbial Science -

### Scope of research

Structure and function of biocatalysis, in particular, pyridoxal enzymes, NAD enzymes, and enzymes acting on xenobiotic compounds are studied to elucidate the dynamic aspects of the fine mechanism for their catalysis in the light of recent advances in gene technology, protein engineering and crystallography. In addition, the metabolism and biofunction of selenium and some other trace elements are investigated. Development and application of new biomolecular functions of microorganisms are also studied to open the door to new fields of biotechnology. For example, molecular structures and functions of thermostable enzymes and their application are under investigation.



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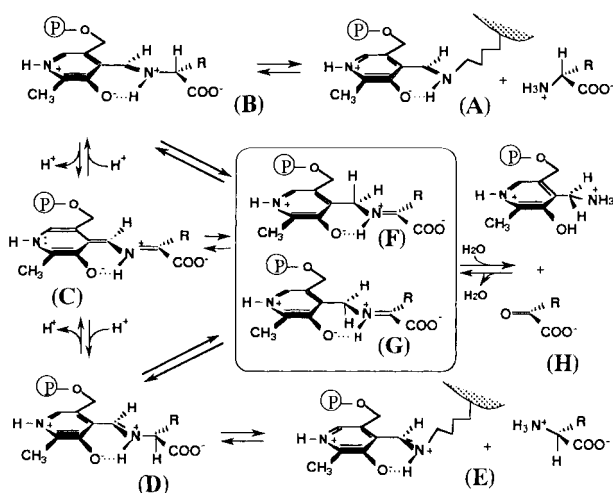
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opposite face of the planar intermediate to that where the proton abstraction occurs, an antipodal aldimine is formed (D). The aldimine complex is subsequently hydrolyzed to form isomerized amino acid, and regenerates the bound PLP (E). The random return of hydrogen to the anionic intermediate is a characteristic of enzymatic racemization among various pyridoxal enzyme reactions. In aminotransferase reactions, the abstracted hydrogen is stereospecifically transferred to C-4' of the cofactor, and a ketimine intermediate is formed. The pyridoxamine 5'-phosphate (PMP) form of the enzyme and a keto acid are produced by hydrolysis of the ketimine intermediate. Amino acid racemases are reported to catalyze the transamination as a side reaction. The transamination catalyzed by amino acid racemases can be attained through a sequence either  $A \rightarrow B \rightarrow F$  (or  $G$ )  $\rightarrow H$ , or  $A \rightarrow B \rightarrow C \rightarrow F$  (or  $G$ )  $\rightarrow H$  (Scheme I). An equivalent route can be delineated for the antipode:  $E \rightarrow D \rightarrow F$  (or  $G$ )  $\rightarrow H$ , or  $E \rightarrow D \rightarrow C \rightarrow F$  (or  $G$ )  $\rightarrow H$ . In transamination, mutual hydrogen transfer between the substrate and C-4' of the cofactor occurs. In all previous studies of transaminations catalyzed by aminotransferases as well as other pyridoxal enzymes, the hydrogen transfer between substrate and cofactor occurs strictly stereospecifically on the *si* or *re* face of the plane of the anionic intermediate. However, if the transamination catalyzed by amino acid racemases proceeds as depicted in Scheme I, the hydrogen transfer should occur non-stereospecifically on both faces of the planar intermediate. We were therefore interested in the stereospecificity for the hydrogen transfer during transamination catalyzed by amino acid racemases as a side-reaction, and provide the first evidence that hydrogen removal from C-4' of PMP occurs randomly on both faces of the substrate-cofactor imine plane during half transamination catalyzed by the amino acid racemases (1). We also show that the enzyme catalyzes non-stereospecific overall transamination between L-ornithine and  $\alpha$ -ketoglutarate as well.

The absorption spectral change has demonstrated that



**Scheme I.** Reaction mechanism of amino acid racemase

the amino acid racemase of broad substrate specificity from *Ps. putida* catalyzes the transamination between PMP and pyruvate. When the PMP-form of an enzyme is converted to the PLP form by transamination with keto acid, one of the two hydrogens at C-4' of PMP is usually transferred stereospecifically to C $\alpha$  of the keto acid. We studied the stereospecificity of amino acid racemase for hydrogen abstraction from C-4' of PMP by measurement of the radioactivity of  $^3\text{H}$  released from the PMPs which are stereospecifically tritiated at C-4' using the method described previously (2). Each 5 nmol of apo-amino acid racemase was incubated with 1 nmol of (4'*S*)- or (4'*R*)-[4'- $^3\text{H}$ ]PMP and 5 nmol of sodium pyruvate. We deduce that PMP was completely converted to PLP because the PMP-form of the amino acid racemase from *Ps. putida* recovered 100 % of the activity theoretically expected. Tritium was released equally from both (4'*S*)- and (4'*R*)-[4'- $^3\text{H}$ ]PMPs in the presence of amino acid racemases. The amount of tritium released from each PMP was about 50% of that which initially existed. The control experiment with D-AAT and AspAT showed that they catalyzed the stereospecific removal of tritium from (4'*R*)- or (4'*S*)-[4'- $^3\text{H}$ ]PMP, respectively. These results confirm the stereospecific tritium labelling of both PMPs. Thus, the amino acid racemases catalyze the non-stereospecific abstraction of hydrogen from C-4' of PMP. They are the first class of pyridoxal enzyme catalyzing the hydrogen removal on both sides of the plane of a substrate-cofactor complex during transamination.

If the hydrogen is introduced non-specifically to C-2 of the keto acid moiety of the anionic intermediate on both sides of the planar intermediate during the half reaction of transamination, racemic amino acid is formed from the keto acid (Scheme I;  $H \rightarrow F$  (or  $G$ )  $\rightarrow C \rightarrow B \rightarrow A$ , or  $H \rightarrow F$  (or  $G$ )  $\rightarrow C \rightarrow D \rightarrow E$ ). We studied the stereochemistry of glutamate formed from  $\alpha$ -ketoglutarate by transamination with L-ornithine catalyzed by the amino acid racemase of *Ps. putida*. After the reaction, the products were derived to diastereomers with Marfey's reagent, and subjected to HPLC. Both enantiomers of glutamate and ornithine were found. The amino acid racemase from *Ps. putida* catalyzes the racemization of ornithine, but glutamate is inert as a substrate for the racemase reaction. Thus, both enantiomers of glutamate were directly formed by transamination, not by racemization of one enantiomer produced through transamination. The amino acid racemase from *Ps. putida* is the first example of a pyridoxal enzyme catalyzing non-stereospecific transamination.

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